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Lab Resource: Stem Cell Line

Generation of human control iPS cell line *CHOPWT10* from healthy adult peripheral blood mononuclear cells

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ABSTRACT

The *CHOPWT10* iPS cell line was generated to be used as a control for applications such as in differentiation analyses to the three germ layers and derivative tissues. Peripheral blood mononuclear cells (PBMCs) obtained from a healthy adult male were reprogrammed using the non-integrating Sendai virus expressing Oct3/4, Sox2, c-Myc, and Klf4.

Resource table: *CHOPWT10*

Name of stem cell construct	*CHOPWT10*
Institution	The Children's Hospital of Philadelphia (CHOP)
Person who created resource	Jean Ann Maguire
Contact person and email	Deborah L. French, frenchd@email.chop.edu
Date archived/stock date	2014
Origin	Human PBMCs
Type of resource	Biological reagent: iPS cell from human PBMCs
Subtype	Cell line
Key transcription factors	Oct4, Sox2, Klf4, c-Myc
Authentication	Identity and purity of cell line confirmed (Figs. 1 and 2)
Link to related literature	N/A
Information in public databases	N/A

Resource details

To generate *CHOPWT10* cells, four Yamanaka reprogramming factors (Oct3/4, Sox2, c-Myc, and Klf4) were delivered into mononuclear cells through Sendai viral infection. The Sendai virus is non-integrating and the absence of the vectors in the reprogrammed cells was confirmed by PCR analysis (Fig. 1A).

DNA fingerprinting confirmed the genetic relation of the derivative line to the parental somatic line (Fig. 1B). The cells exhibited normal karyotype (46, XY) upon G-band analysis (Fig. 1C).

Pluripotency was verified by gene expression of pluripotent stem cell markers Oct4, Sox2, Nanog, Rex1, DMNT3B and ABCG2 by qPCR (Fig. 1D). In addition, pluripotent surface marker expression at single cell resolution of SSEA3, SSEA4, Tra-1-60, and Tra-1-81 were confirmed by flow cytometry (Fig. 1E). Differentiation capacity into three germ layers was confirmed by PluriTest™ and quantitative PCR (Fig. 2).

Materials and methods

Blood sample collection and processing

Blood samples were collected from a healthy adult male into BD Vacutainer cell preparation tubes. The PBMCs were isolated by centrifugation at 1800 rcf for 30 min, after which mononuclear cells were transferred to a fresh tube, washed with PBS, and pelleted at 300 rcf for 10 min. Pellets were resuspended in freezing media (90% FBS, 10% DMSO) and stored in liquid nitrogen. Blood collection was approved by the CHOP Institutional Review Board committee with written informed consent.

iPS cell reprogramming

The PBMCs were expanded and reprogrammed as previously described (Yang et al., 2012). Briefly, PBMCs were expanded in QBSF-60 medium containing EPO (2 U/mL), IGF-1 (40 ng/mL), SCF (50 ng/mL), IL-3 (10 ng/mL), dexamethasone (1.5 μM), ascorbic

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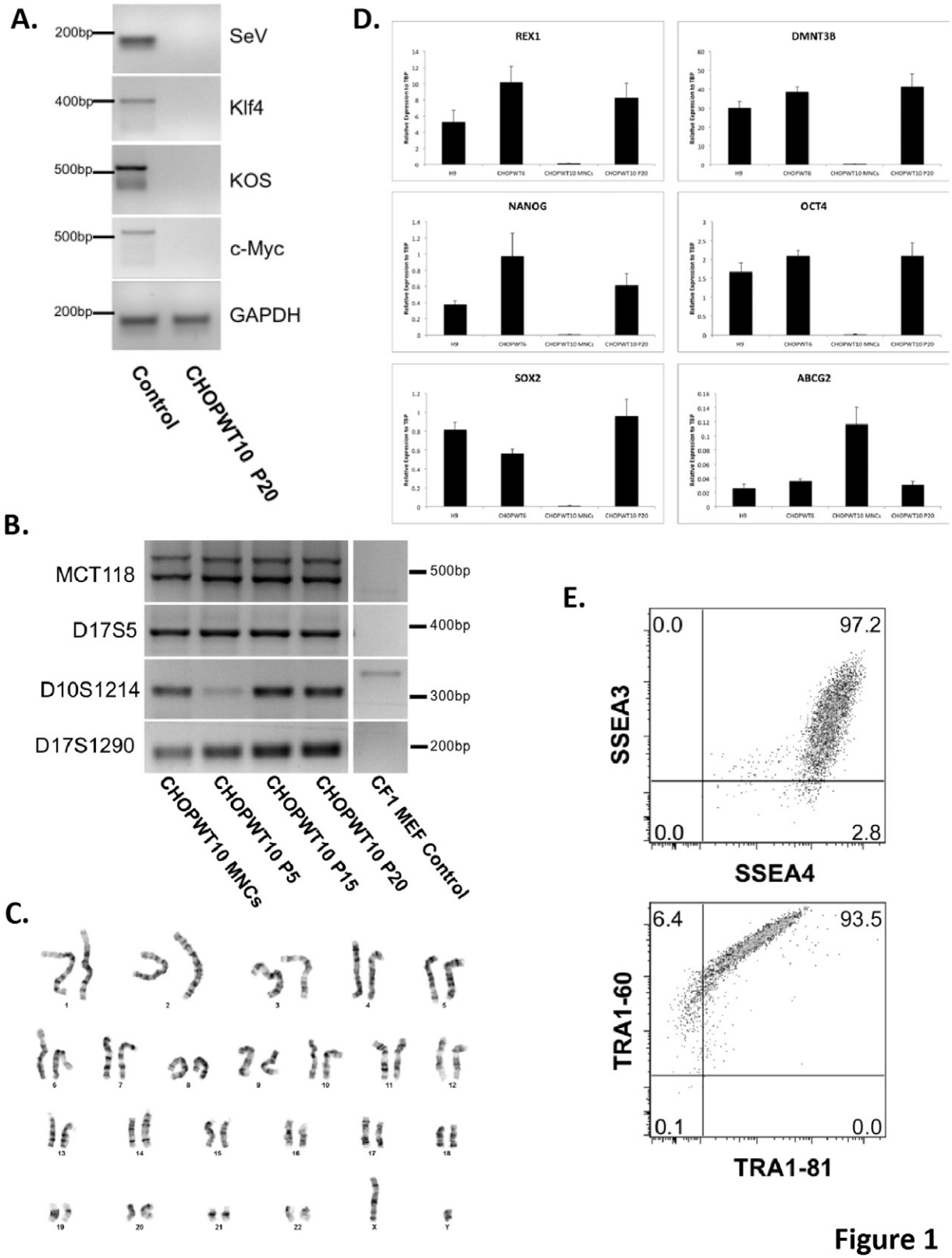


Figure 1

Fig. 1. *Generation and characterization of CHOPWT10.* (A) PCR analysis of reprogramming genes after 20 passages of *CHOPWT10* using GAPDH as a loading control. Cells harvested immediately post-Sendai infection were used as a positive control. (B) *CHOPWT10* iPS cells compared to parental cells by DNA fingerprinting. (C) Karyotype analysis of *CHOPWT10*. (D) Quantitative PCR of relative gene expression of pluripotency genes compared to embryonic stem cells (H9) and a control iPS cell line (CHOPWT6). (E) Flow cytometry profiles of surface markers on undifferentiated cells: SSEA3, SSEA4, TRA-1-60, and TRA-1-81.

acid (50 ng/mL), glutamine (1%), and penicillin/streptomycin (1%) for 7 days. Cells were transduced with Sendai virus expressing human Oct3/4, Sox2, Klf4, and c-Myc. (Life Technologies) (moi according to manufacturer's instructions). Transduced cells were plated on culture dishes containing irradiated mouse embryonic fibroblasts (MEFs), and maintained in human embryonic stem

cell media (HES) containing 5 ng/mL of bFGF. The medium was replenished every 2–3 days for 3 weeks. Cells were maintained in these conditions until uniform colonies were generated, and iPS cell colonies were mechanically isolated and expanded on MEFs.

DNA fingerprinting

Confirmation of the genetic integrity of the iPS cell line to the parental line was established by DNA fingerprinting. PCR amplification was performed on genomic DNA isolated from parental and iPS cells with reference primer sets (Ban et al., 2011; Park et al., 2011; Dirks et al., 1999). Products were separated on a 3.0% agarose gel and visualized with ethidium bromide.

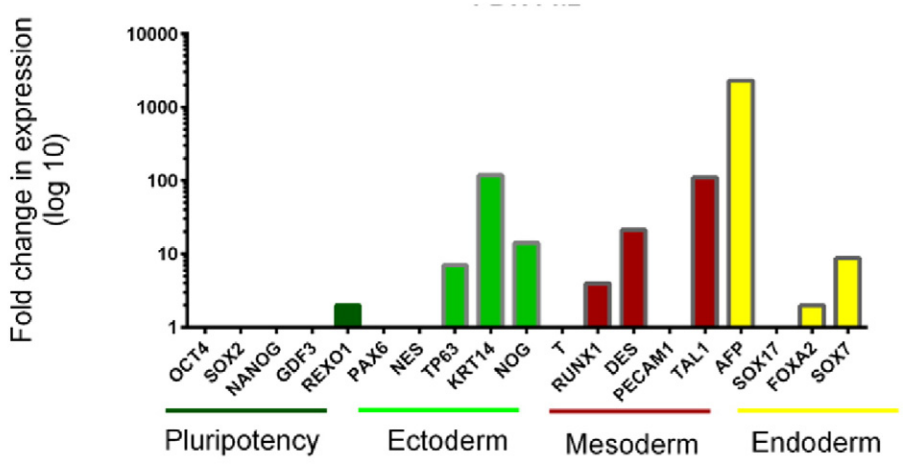
Karyotype analysis

Chromosomal G-band analyses were performed by Cell Line Genetics (Madison, WI).

RT-PCR and quantitative PCR

RNA was isolated from the *CHOPWT10* iPS cells using the RNeasy micro kit (Qiagen) according to the manufacturer's protocol. Reverse transcription was performed using random hexamers with Superscript III Reverse Transcriptase (Life Technologies) and 500 ng total RNA. cDNA was used as a template for PCR amplification of the viral backbone and exogenous reprogramming factors (SeV, Klf4, Oct3/4, Sox2, and c-Myc). Primer sequences were provided by the company (Life Technologies). PCR products were separated on a 2.0% agarose gel and visualized with ethidium bromide. RT-qPCR was performed on a LightCycler-480II (Roche, IN). All experiments were performed in triplicate using SYBR-Green qPCR supermix (Roche) according to the manufacturers instructions. Primers for all endogenous genes were prepared as previously described (Mills et al., 2013). Human genomic DNA (gDNA), diluted in 10-fold increments from 1 to 100 ng/μL, was used for the qPCR standard curve to determine PCR efficiency and relative gene expression compared to the housekeeping gene TBP (TATA Binding Box Protein).

A.



Gene	Fold change	Gene	Fold change	Gene	Fold change	Gene	Fold change
OCT4	0	PAX6	0	T	1	AFP	2302
SOX2	0	NES	0	RUNX1	4	SOX17	1
NANOG	0	TP63	7	DES	21	FOXA2	2
GDF3	0	KRT14	117	PECAM1	0	SOX7	9
REXO1	2	NOG	14	TAL1	109		

B.

Sample ID	Pluripotency Score	Novelty Score
H9-1	39.9	1.32
H9-2	38.9	1.30
CHOPWT10	36.0	1.28

Fig. 2. *Pluripotency potential of CHOPWT10.* (A) Fold change in expression of pluripotency genes and genes specific to each germ layer compared to undifferentiated cells. (B) PluriTest™ results using embryonic stem cells (H9) as a control.

Flow cytometry analysis

Expression of pluripotency markers were evaluated by flow cytometry using the following antibodies: Alexa-Fluor®-647 α-human SSEA4 (1:400) and Tra-1-681 (1:200); Alexa-Fluor®-488 SSEA3 (1:200) and Tra-1-60 (1:200) (BioLegend). Accutase-dissociated single cells were analyzed using a FACSCanto flow cytometer (BD Biosciences) and the FlowJo software program (Tree Star, CA).

Pluripotency potential

PluriTest™ was performed as previously described (Muller et al., 2011) using Illumina HT12 array and the PluriTest Software (www.pluritest.org) by the Coriell Institute for Medical Research. Pluripotent cells have a Pluripotency Score greater than 20 and Novelty Score less than 1.62. Embryonic stem cells (H9) were used as a control and each sample was run as a biological replicate. To measure the capacity to form three germ layers, *CHOPWT10* cells were differentiated using embryoid body formation. Gene expression was measured by quantitative RT-PCR using the endogenous housekeeping gene, GAPDH. Pluripotency was confirmed by two-fold or higher expression of at least one gene specific to each germ layer compared to undifferentiated cells.

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